Influence of Sulfur and Boron on the growth and yield of Broccoli

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Abstract— *Field experiment was conducted to study the impact of sulfur (S) and boron (B) on yield and yield component of broccoli. Sulfur was applied @ 0 (control), 20 and 40 kg ha^{-1} as elemental sulfur while B was applied at the rate 0, 1 and 1.5 kg ha^{-1} as borax along with a basal dose of N,P and K @ 120, 90 and 60 kg ha^{-1}. All the fertilizers were applied at the time of sowing. The experimental design used was randomized complete block design (RCBD) with three replications. The data on plant height, number of leaves, flower diameter, head yield and biological mass were recorded along with S and B concentration in soil after crop harvesting. The result revealed that yield and yield parameter increased with increasing levels of S and B with higher head yield, flower diameter and plant height were observed when 40 kg ha^{-1} S and 1.5 kg ha^{-1} B were applied. It was further noted that head yield and head diameter were non-significant when averaged across the B treatment between 20 and 40 kg ha^{-1} applied S but significant from control. Similarly, when the yield parameters were average across the S treatment, there was a significant and linear increase with higher B level. Soil analysis showed that both B an S concentration in soil increased by increasing level of applied S and B. So the optimum level of S and B for broccoli was 40 and 1.5 kg ha^{-1}respectively for higher yield of broccoli.*

Keywords— ***Boron, broccoli, growth and yield, Sulfur.***

I. INTRODUCTION

Broccoli (*Brassica oleraceaitalica)*is a green edible vegetable in the cabbage family, whose flower head is utilized for cocking. Its cultivation originated in Italy. Broccolo, its Italian name, means "cabbage sprout". Because of its different components, this vegetable provides a complex of tastes and textures, ranging from soft and flowery (the florets) to fibrous and crunchy (the stem and stalk). Its color can vary as deep sage, dark green and purplish-green, depending upon the variety [**1**, **2**]. Broccoli is better adapted to tolerate comparatively high temperature [**3**]. Unlike cauliflower, broccoli produces smaller flowering shoot from the leaf axile if the main apical flower bud is removed. Consequently a field of broccoli may be harvested over a considerable period of time. This food is very low in saturated fat and cholesterol. It is also a good source of protein, vitamins (A, C, E, K and B6), thiamin, riboflavin, pantothenic acid, calcium, iron, magnesium, phosphorus, selenium, dietary fiber, folate, potassium and manganese. Broccoli also contains large concentrations of caroteniods, which have preventive qualities with regards to human cancer [**4**,**5**]demonstrated that fresh broccoli heads contain relatively high levels of glucosinolates and flavonoids that may be chemoprotective against human cancer. Broccoli is a cool-weather crop that does poorly in hot summer weather. It grows best when exposed to an average daily temperature between 18 - 23 °C [**6**].It is an important vegetable crop and has high nutritional and good commercial value [**7**].Nowadays, broccoli attracted more attention due to its diverse use and great nutritional value [**8**,**9**]. Broccoli is known as the “Crown jewel of nutrition” for its vitamin rich, high in fiber, and low in calorie properties [**10**].

Broccoli is grown all over the world. In 2012, total production all over the world was 21,266,789tons. China produced maximum broccoli (9,596,000 tons) followed by India (7,000,000 tons) while Pakistan was at number eight in broccoli production by producing 224,000 tons in 2012[**11**]**.** Successful production of broccoli depends on various factors of which

fertilizer application is the most important one. Generally, large amount of inorganic fertilizers are applied to vegetable for higher yield and for maximum growth [**12**,**13**].

Sulfur, an essential plant nutrient, accounts for 0.2 to 0.25% (20 to 50g kg^{-1}) of plant dry matter. It is rapidly incorporated into organic molecules, the first stable organic S-compound being cysteine, and methionine, are the most important S-containing amino acid in plants which serve as building block of protein[**14**].In plants, about 90% amino acids contain sulfur. It is a primary constituent of protein because three amino acids (cysteine, cystine and methionine) contain sulphur. It helps in formation of chlorophyll. It also helps in synthesis of oil and is therefore very much essential for oil seed crops. It keeps enzymes active and helps in regulation of biochemical reaction. It increases crop yield and improves produce quality [**15**]. Sulphur is present in soil in organic and inorganic forms. The organic S accounts for more than 95% of the total S in most soils of humid and semi humid regions [**16**]**.**The quantity of organic and inorganic S in a soil sample changes usually according to soil type and depth of sampling. In poorly drained or water logged soils, the main forms of inorganic S in soils is sulfide and often elemental S [**17**]. Major factors affecting forms of sulphur are organic matter, texture, climate, altitude, salt content, vegetation, leaching, cropping intensity, flooding and carbonates. Transformation of added S is more complex when organic or elemental/sulfide sources are used as compared to sulfate sources.

Sulphur fertilizers help to enhance the uptake of N, P, K, and Zn in plants. Due to the synergistic effect of S on these elements, their efficiency is enhanced which result in increased crop productivity [**16**]. Sulphur fertilization is a feasible technique to suppress the plant uptake of undesired toxic elements because of the antagonistic relationship between S and other elements including Mg, Mo, and Se. Thus S fertilizers are not only useful in increasing crop production and quality of produce but also in improving soil condition for crop growth.

Boron is one of the recognized micro nutrients for plant growth and production. Boron is essential for plant growth and development. It is known to play role in cell division, water relations, ion absorption, IAA and carbohydrates metabolism, translocation of sugars, fruit and seed development and its deficiency may affect all these processes [**18**,**19**]. Its application to the soil increased head yield of broccoli [**20**]. A great deal of attention has been given to studying the effect of B supply on behavior of cations such as K^+, Ca^{++}, Mg^{++} and Na^+ in various crop species, but information on the behavior of anions, specially phosphorous, is limited. The early work of [**21**] indicated that B deficiency in corn and broad beans reduced the capacity for the absorption of PO_4 due to the reduced ATPase activity, which could be rapidly restored by the addition of boron. Later on [**22**]reported that B plays role in biochemical process in plants e.g. carbohydrates metabolism and transport of sugar through membranes, nucleic acids (DNA and RNA) synthesis, tissue development and formation of cell walls. All these functions are closely related to phosphorous. Therefore, it can be assumed that the borate ions may be fixed in the cell wall as an organic complex and would be responsible for a change in the permeability of the cell wall, resulting in a reduction of phosphate uptake and carbohydrates, under the deficient and toxic conditions of added boron. It deficiency causes many anatomical, physiological and biological changes. Hollow stem disorder is a major problem for broccoli production and is commonly associated with B deficiency [**23**].

Among the essential micronutrients, boron and molybdenum play vital role in developing the economic plant parts of crop plants. Due to boron deficiency water soaked areas appear on the stem and head surface. Gradually the stem becomes hollow and curd turns brown. Again the molybdenum deficiency appears on young plant with chlorosis of leaf margins and gradually the whole leaf turns white. As a result the leaf blade fails to develop properly and only the midrib portions develop resulting sword like appearance of leaves giving whiptail symptom. Besides, the quantity of boron and molybdenum depend on soil type, soil reaction and the extent of deficiency [**24**].

II. MATERIALS AND METHODS

The present research was conducted during the winter season using broccoli (*Var premium*) as a test crop to determine the influence of sulfur and boron on the growth and yield of broccoli in Agriculture Research Institute, Khyber Pakhtunkhwa. There were nine treatments combinations consisting of different levels of B and S along with basal doses of N, P, and K as given in (**Table 1**). All S and B were applied in bands at the time of sowing. The experiment was laid out in a randomized complete block design with three replications. After seven days of fertilizer application, 25-days old seedlings of Broccoli were transplanted to a 3x2 m^2 plot with 60 cm row to row and 45cm plant to plant distance. For proper seedling establishment, irrigation was given for seven days after transplanting. Other culture practices were adopted as and when required. The data on plant height, number of leaves per plant were recorded in each plot. Secondary heads were taken and

marketable head yield was recorded at each harvest per plot in grams and was converted into yield in tons per hectare. Data on head diameter and marketable head weight per plant (terminal + lateral heads) was recorded in each plot. Six core samples were collected from each plot with the help of core sampler at a depth of 0-30 cm before and after harvesting. The samples from each plot were composted, properly packed, labeled and were brought to the laboratory of Soil Fertility Section, Agricultural Research Institute, Mingora, Swat. The samples were air-dried, ground, passed through 2mm sieve and were stored in the laboratory for the following physico-chemical analysis, including pH, EC, texture, lime, organic matter, AB-DTPA extractable phosphorus and total nitrogen. After harvesting, each plot was sampled separately and analyzed for boron and sulfur. Soil texture was determined by hydrometer method [**26**]. Electrical conductivity (EC) of soil was determined in soil water (1:5) suspension. Ten g soil sample was shaken with 50 mL distilled water for 30 minutes. After filtering, EC of the extract was determined with the help of EC meter [**27**]. Soil pH was determined in soil water (1:5) suspension. Ten g soil sample was shaken with 50 mL distilled water for 30 minutes. After filtering, pH of the extract was read for pH with the help of pH meter using combined hydrogen and calomel electrod[**28**].

Organic matter in soil was determined by the modified method [**29**]. Soil total N was determined calorimetrically, following the Kjeldahl procedure[**17**].AB-DTPA extractable P and K were measured in the soil by [**30**].Fifty mL 0.001 M $CaCl_22H_2O$ reagent was added to a 25g soil sample then shaken for 30 minutes and was filtered by Whatman 42 No filter paper. One mL extract was taken in a 25 mL volumetric flask. Five mL mixed acid reagent and 1 mL acid sulfate were added, and was mix thoroughly. Let it to stand for three minutes. Then a 0.5 g $BaCl_2$-$2H_2O$ fine powder was added. After this mL gum acacia reagent was added and was mixed again thoroughly and SO_4-S was determined on spectrophotometer with standard solutions at [**31**]. The concentration of extractable B in soil was determined by dilute HCl procedure.

TABLE 1
EXPERIMENTAL TREATMENTS OF DIFFERENT FERTILIZERS.

Treatments	N (kg ha^{-1})	P_2O_5(kg ha^{-1})	K_2O (kg ha^{-1})	S (kg ha^{-1})	B (kg ha^{-1})
T_1	120	90	60	0	0
T_2	120	90	60	20	0
T_3	120	90	60	40	0
T_4	120	90	60	0	1
T_5	120	90	60	0	1.5
T_6	120	90	60	20	1
T_7	120	90	60	40	1
T_8	120	90	60	20	1.5
T_9	120	90	60	40	1.5

N= Nitrogen, P_2O_5 = Phosphorous penta oxide, K_2O = Potassium dioxide, S= Sulfur, B= Boron.

2.1 Statistical analysis

Statistical Replicated data were analyzed using Statistix8.1 software using factorial analysis. Means were compared using least significant difference (LSD) test [**32**].

III. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Physico-chemical characteristics of the experimental soil

The results of physic-chemical characteristics of soil sample collected for 30 cm depth and its evaluation for fertility status are shown in Table 2.Physico-chemical characteristics of experimental site showed that the soil was silt loam, slightly acidic (6.0) non saline, slightly calcareous (2.6) and marginal in organic matter(1.18%).

TABLE 2
PHYSICO CHEMICAL ANALYSIS OF THE EXPERIMENTAL FIELD

Physico chemical analysis	Unit	Value
Texture	-	Silt loam
pH	-	6.0
EC	dSm^{-1}	0.16
Organic matter	%	1.11
Lime Content	%	2.60
P	$mgkg^{-1}$	5.05
K	$mg\ kg^{-1}$	37.5
Total N	%	0.02
Mineral N (NH_4+NO_3-N)	mg kg-^{1}	16.63
SO_4-S	$mg\ kg^{-1}$	11.3
Boron	$mg\ kg^{-1}$	0.26

EC= Electrical conductivity, P= Phosphorous, K= Potassium, N= Nitrogen, So_4-S = Sulfate sulfur

Fertilility evaluation of the soil reveled that it was deficient in total N (0.028%), K(37.5mg kg^{-1}) B(0.26 mg kg^{-1}) whereas marginal in available SO_4-S(11.3 mg kg^{-1}) and P (5.50 mg kg^{-1}). Ali (2008) reported similar physic-chemical characterization of soil while working on sulfur mineralization in important soil series of Khyber Pakhtunkhwa.

3.1.2 Yield and yield parameter Data

3.1.2.1 Plant Height (cm)

When the plant height values were averaged (Table 3) across the sulfur treatments, there was a significant and linear increase in plant height with increasing B application being significantly ($p<0.05$) higher when higher level of B was applied. Similarly, when the values were averaged cross the B treatment there was significant plant height increased with the addition of Sulfur. However, there was no significant difference between the two S treatments while significantly deficient from control where no sulfur was applied. The interaction of S and B was non-significant even though comparatively higher plants were noted where higher B and S doses were applied compared to control.

TABLE 3
PLANT HEIGHT, NUMBER OF LEAVES, FLOWER DIAMETER, BIOLOGICAL YIELD, HEAD YIELD OF BROCCOLI AS AFFECTED BY B AND S APPLICATION.

Boron (kg ha^{-1})	Plant height (cm)	Number of leaves	Flower Diameter(cm).	Biological yield (t ha^{-1})	Head yield (t ha^{-1})
0	30 ^{c}	15.44 ^{c}	24.91 ^{c}	132.89 ^{c}	18.05 ^{c}
1	32.58 ^{b}	16.89 ^{b}	26.40 ^{b}	164.04 ^{b}	20.69 ^{b}
1.5	35.70 ^{a}	17.56 ^{a}	28.42 ^{a}	215.77 ^{a}	23.08 ^{a}
LSD $_{0.05}$	0.68	0.60	0.42	9.58	1.4
Sulfur (kg ha^{-1})					
0	31.96 ^{c}	15.44 ^{c}	25.17 ^{b}	160.04 ^{b}	19.25 ^{b}
20	32.80 ^{b}	16.78 ^{b}	27.37 ^{a}	172.90 ^{a}	21.33 ^{a}
40	33.85 ^{a}	17.67 ^{a}	27.18 ^{a}	179.76 ^{a}	21.24 ^{a}
LSD $_{0.05}$	0.68	0.60	0.42	9.58	1.4
Interaction					
B x S	NS	NS	NS	NS	NS

Means in similar category of columns and rows with different alphabets(superscripts) differ significantly from each other at p<0.05 using LSD,NS= non significant. B= Boron, S= Sulfur.

3.1.2.2 Number of leaves plant^{-1}

When the number of leaves plant^{-1} values were averaged (**Table 3**) across the sulfur treatments, there was a significant and linear increase in number of leaves plant^{-1} with increasing B application being significantly ($p<0.05$) higher when higher level of B was applied. Similarly, when the values were averaged cross the B treatment there was significant number of leaves plant^{-1} increased with the addition of Sulfur. However, there was no significant difference between the two S treatments while significantly deficient from control where no sulfur was applied. The interaction of S and B was non-significant even though comparatively higher number of leaves plant^{-1} were noted where higher B and S doses were applied compared to control.

3.1.2.3 Flower diameter (cm)

When the head diameters values were averaged (**Table 3**) across the sulfur treatments the results, there was a significant and linear increase in head diameter with increasing B application being significantly ($p<0.05$) higher when higher level of B was applied. Similarly when the values were averaged across the B treatment there was significant head diameter with the addition of Sulfur. However, there was no significant difference between the two S treatments while significantly deficient from control where no sulfur was applied. The interaction of S and B was non-significant even though comparatively broader flower were noted where higher B and S doses were applied compared to control.

3.1.2.4 Head yield (tha^{-1})

When the head yield values were averaged across (**Table 3**) the sulfur treatments, there was a significant and linear increase in head yield with increasing B application being significantly ($p<0.05$) higher when higher level of B was applied. Similarly, when the values were averaged cross the B treatment there was significant head yield increased with the addition of Sulfur. However, there was no significant difference between the two S treatments while significantly deficient from control where no sulfur was applied. The interaction of S and B was also significant. Higher head yield were noted where higher B and S doses were applied compared to control.

3.1.2.5 Biological Yield (t ha^{-1})

When the biological yield values were averaged (**Table 3**) across the sulfur treatments, there was a significant and linear increase in biological yield with increasing B application being significantly ($p<0.05$) higher when higher level of B was applied. Similarly, when the values were averaged cross the B treatment there was significant biological yield increased with the addition of Sulfur. However there was significant difference between the two S treatments while significantly deficient from control where no sulfur was applied. The interaction of S and B was non-significant even though comparatively higher biological yield were noted where higher B and S doses were applied compared to control.

3.1.2.6 Concentration of Boron in soil after harvesting

Boron concentration in soil after harvesting broccoli is presented in table 5. Statistical analysis of the data revealed that B application has significantly ($p<0.05$) increased its concentration in soil. In case of B application maximum concentration 1.27 kg ha^{-1} was recorded when 1.5 kg B ha^{-1}was applied while minimum 0.35 kg ha^{-1} was obtained in control having no B. Interaction of B*S on concentration of boron in soil after harvesting was also statistically significant. Maximum concentration was found by B_3*S_2 (1.43 kg ha^{-1}) followed by B_3 * S_3 (1.04 kg ha^{-1}) while minimum was obtained by where neither B nor S was applied.

3.1.2.7 Concentration of Sulfur in soil after harvesting

Table 4 shows the data pertaining to the concentration of sulfur in soil after harvesting Statistic analysis of the data revealed that S had significantly ($p<0.05$) affected the concentration of sulfur. In case of S application, maximum concentration (43.31 kg ha-1) was found in S3 and minimum concentration (12.16 kg ha-1) was found in S1. The addition of B significantly reduced S concentration.

TABLE 4
CONCENTRATION OF BORON AND SULFUR IN SOIL AFTER HARVESTING

Boron (kg ha^{-1})	Concentration of B	Concentration of S
0	0.35^{c}	25.68^{a}
1	0.82^{b}	26.51^{a}
1.5	1.27^{a}	24.464^{b}
LSD $_{0.05}$	7.78	0.86
Sulfur (kg ha^{-1})		
0	0.83^{b}	12.16^{c}
20	0.87^{a}	21.18^{b}
40	0.74^{c}	43.31^{a}
LSD $_{0.05}$	7.78	0.86
Interaction		
B x S	NS	NS

Means in similar category of columns and rows with different alphabets (superscripts) differ significantly from each other at p<0.05 using LSD-NS= non significant.

3.2 Discussion

Data presented in table 4 revealed that B and S had significant positive effect on plant height of broccoli while interaction between boron and sulfur was statistically non-significant. Increased plant height was recorded by treatment receiving boron and sulfur at the rate of 1.5 kg ha^{-1} and 40 kg ha^{-1} respectively. Enhanced carbohydrates metabolism, formation of cells and tissue development due to applied boron and increased photosynthetic activities due to S might have caused increase in plant height. These results are in agreement with **Azza** [**23**].

Increased photosynthetic activities and enhanced uptake of nutrients due to sulfur, and improved metabolic activities due boron might have resulted in more leaves per plant. These results confirmed by earlier work **Cakmak** [**24**]. Results of the present study are also in agreement with **Yang Xian** [**20**]who found that S,B, Zn and Mo had significant effects on growth and yield parameters like numbers of leaves per plant, head weight of broccoli.

The data revealed that head diameter was significantly affected by B application at $p < 0.05$. However, effect of S alone and the interaction between B and S was non-significant. Higher diameter was obtained by plot receiving 1.5 kg B ha^{-1} and 20 kg S ha^{-1}. This increase in head diameter might be attributed to the role of B and S in much biochemical process within the plants like carbohydrates metabolism, transport of sugar through membranes, tissue development, formation of cell walls, help in cell division, and sulfur increase in chlorophyll content which increases photosynthetic activities. These results agree with **Moniruzzaman**[**26**]who studied that S,B, Zn and Mo had significant effects head diameter, main head weight of broccoli. The finding of present study is also supported by **Shelp** [**20**]who found that Mo and B application significantly increased head diameter, weight and yield of cauliflower.

Data presented in **Table 4** revealed that B, S and their interaction had significant ($p<0.05$) effect on head yield of broccoli. Optimum yield was obtained by treatment receiving 1.5 kg B ha^{-1} and 20 kg S ha^{-1}. This might be due the fact that B plays roles in many biochemical process in plants like carbohydrates metabolism and transport of sugar through membranes, tissue development and formation of cell walls and help in cell division. Sulfur increases chlorophyll content which improves photosynthetic activities [**34**,**17**]. These results agree with **Yang Xiang**[**15**] who observed that application of nitrogen and boron at specific rate increased head yield and quality of broccoli. Similarly **Ali** [**22**] also obtained significantly maximum head yield in broccoli by the application of boron. These results also agree with **Bharathi** [**35**] who reported that S,B Zn and Mo had significant effects on growth and yield parameters viz. plant spread, numbers of leaves per plant, head diameter and main head weight of broccoli.

The data revealed that B and S and has significant ($p<0.05$) positive effect on biological yield of broccoli while interaction between boron and sulfur was statistically non-significant. Optimum biological yield was obtained by treatment receiving Boron at the rate of 1.5 kg ha^{-1} and Sulfur at the rate of 20 kg ha^{-1}. This might be due the biochemical function of boron in plants is carbohydrates metabolism and transport of sugar through membranes, tissue development and formation of cell walls and help in cell division and sulfur increase chlorophyll content which increases photosynthetic activities. These results

agree with **Aulakh**[**36**]who observed that application of nitrogen and boron at specific rates increased wet bio mass, head yield and quality of broccoli.

Data regarding on boron concentration in soil after harvesting of Broccoli are presented in table 8. Statistical analysis of the data revealed that both B has significantly ($p<0.05$) affected the concentration in soil. In case of B maximum concentration was obtained by B3 (1.2778 kg ha^{-1}) while minimum was obtained by B1 (0.3511 kg ha^{-1}). Interaction B * S on concentration of boron in soil after harvesting of broccoli was statistically significant ($p<0.05$). The maximum concentration was found by B3 * S2 (1.4300 kg ha^{-1}), followed by B3 * S3 (1.043 kg ha^{-1}) while the minimum was obtained by B1 * S1 (.2700 kg ha^{-1}).

Data regarding on the concentration of sulfur in soil after harvesting of Broccoli are presented in table 8. Statistical analysis of the data revealed that S had significantly ($p<0.05$) affected the concentration of sulfur in soil. In case of S, maximum concentration was found in S3 (43.313 kgha^{-1}) and minimum concentration was found in S1 (12.162 kg ha^{-1}).) Interaction effect of boron and sulfur (B * S) on concentration of sulfur in soil of broccoli was statistically significant ($p<0.05$). The maximum yield was obtained by B2 * S3 (44.517 kg ha^{-1}), followed by B3 * S3 (41.57 kg ha^{-1}) while the minimum was obtained by B1 * S1 (11.787 kg ha^{-1}).

IV. CONCLUSION

From the present findings of this study summarized above in results it is concluded that: Boron and sulfur enhance the production of broccoli. Optimum yield of broccoli was obtained when sulfur was applied at the rate of 40 kg ha^{-1} and boron at the rate of 1.5 kg ha^{-1}.For optimum yield, 40kg S ha^{-1}and 1.5 kg B ha^{-1} is recommended dose for obtaining optimum yield of broccoli in agroclimatic region of Khyber Pakhtunkhwa Pakistan.

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